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The Gametophyte of *Lycopodium deuterodensum* – Type II or I

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ABSTRACT.—The spores of *Lycopodium deuterodensum* germinate after 3 weeks in the dark on a nutrient medium containing inorganic nutrients and glucose. The dark grown prothalli have the characteristics associated with Type I and II gametophytes – a ring meristem, radial symmetry, and lack paraphyses and photosynthetic lobes. The younger gametophytes have the carrot shape of a Type II gametophyte with a tapering base, a constricted neck, and a gametangial cap with antheridia. With additional growth, the gametophytes become as wide as long and finally wider than long. The wider than long gametophytes are the first to have both antheridia and archegonia on their gametangial caps. The largest gametophytes grown in culture are Type I with irregular disk shapes. The antheridia on all gametophytes are sunken and, for the Lycopodiaceae, the archegonia have medium sized necks with only 3–4 neck canal cells. Although the specific type of gametophyte has not been determined for this species, those grown in culture have the characteristics recognized for subterranean, nonphotosynthetic, mycorrhizal gametophytes of the Lycopodiaceae.

The sporophyte of *Lycopodium deuterodensum* Herter is a large terrestrial lycopod from the South Pacific. It is branched with numerous cones and may attain a meter in height. The gametophyte of this species is undescribed. However, a young sporophyte may have provided an indication of the general type of gametophyte for this species. Holloway (1910, 1916) reported a sporeling of *L. densum* Labill. (= *L. deuterodensum*) with a large subterranean foot and he concluded that the gametophyte of this species was subterranean and long lived.

Because the gametophyte of this species, the sole representative of Section *Pseudolycopodium* (Øllgaard, 1987), has not been collected from natural conditions, this study was carried out using the techniques of axenic culture. Gametophyte growth in culture would provide material for morphological investigations. Determinations could then be made on the correctness of Holloway's conclusion and the type of *Lycopodium* (*sensu lato*) gametophyte (Bruchmann, 1898) in *L. deuterodensum*.

MATERIALS AND METHODS

Spores of *Lycopodium deuterodensum* Herter were obtained from plants in New Caledonia and New Zealand. The system of classification followed in this

report is that of Øllgaard (1987; 1989). The spores were sown within one month of their collection. They were surface sterilized with 20% Clorox (1.1% sodium hypochlorite) by the method of Whittier (1964), collected on sterile filter paper, suspended in sterile water, and sown on 14 ml of nutrient medium in culture tubes (20 × 125 mm) with screw caps that were tightened to reduce moisture loss. The sown spores were maintained in darkness or under a 14 hour photoperiod (50 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) under Gro-lux fluorescent lamps at 22±1°C.

The nutrient medium contained, as a final concentration per liter, 50 mg $MgSO_4 \cdot 7H_2O$, 20 mg $CaCl_2$, 50 mg K_2HPO_4, and 100 mg NH_4Cl or NH_4NO_3. The mineral components of the medium were completed with 0.25 ml of a minor element solution (Whittier and Steeves, 1960) and 4 ml of a FeEDTA solution (Sheat et al., 1959). The medium was solidified with 1.0% agar and was at pH 6.0 prior to autoclaving. The carbon source was provided by the addition of 2.5 g of glucose per liter for spore germination and early gametophyte growth or 5 g per liter for the growth of older gametophytes.

To determine the percentage of spore germination, 400 or more spores were examined. The sample size for calculating the average sizes of the gametangia was 30.

The gametophytes were fixed with Randolph's modified Navashin fluid (CRAF; Johansen, 1940). After fixation, the gametophytes were embedded in paraffin and sectioned by conventional techniques (Johansen, 1940). The sections were stained with Heidenhain's hematoxylin, safranin O, and fast green.

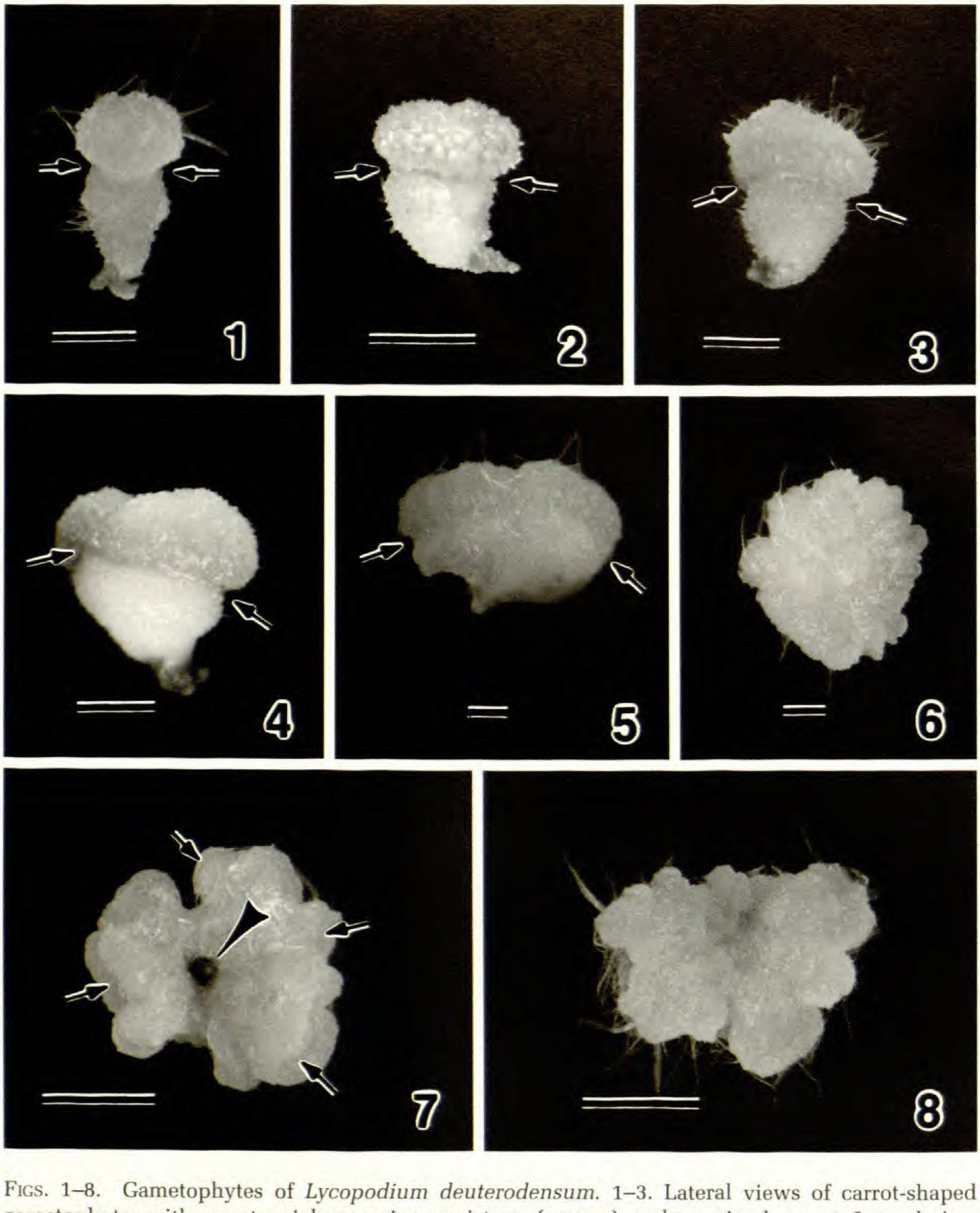
Results

After three weeks in the dark, 2.4% of the spores germinated. Germination was initiated during the third week because no germination had occurred on day 14. At one month, 12% of the spores had germinated. There was no germination in illuminated cultures after 9 months.

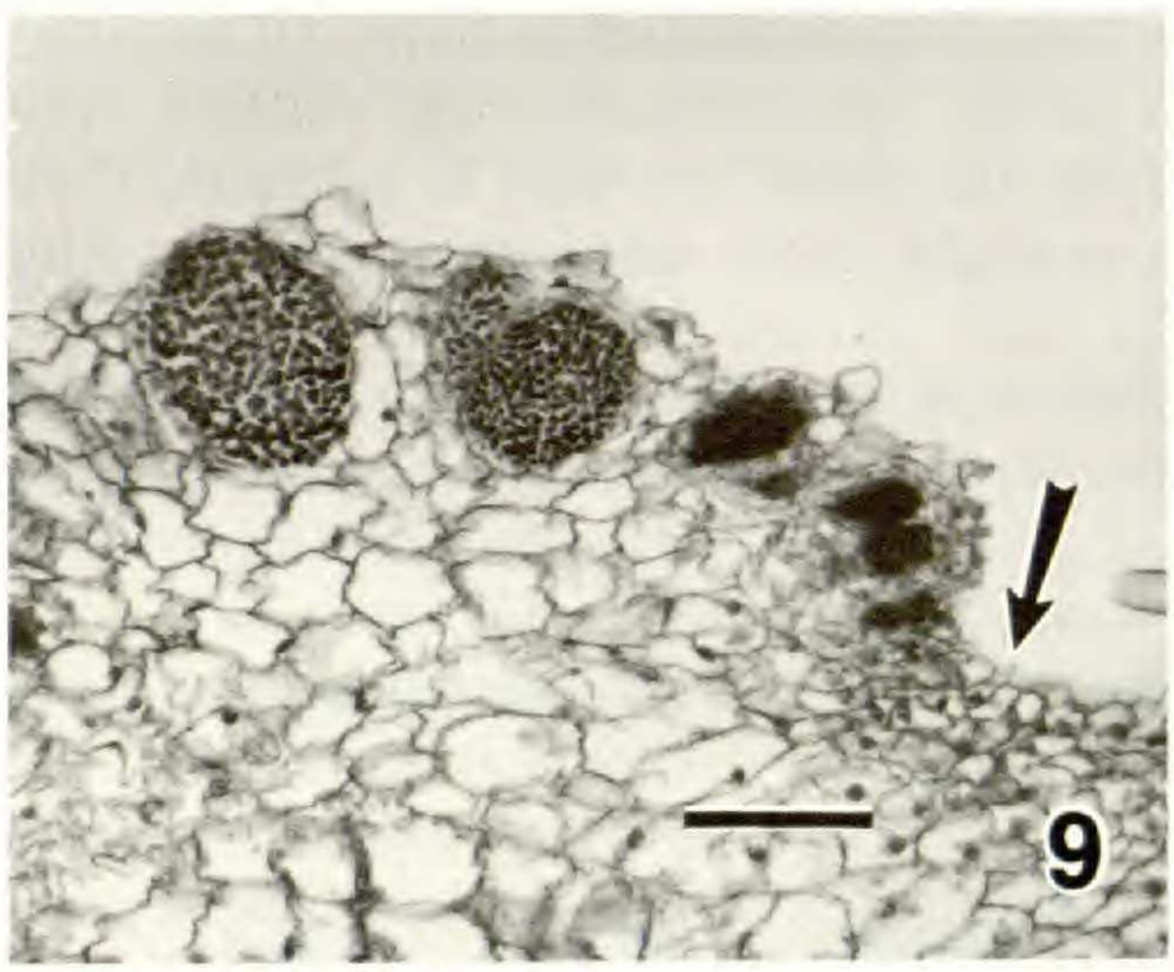
Small gametophytes with antheridia were present at six months. Each of these gametophytes had a tapering base with rhizoids, ring meristem, and gametangial cap with antheridia. The smallest were 1 mm long. They were small carrot-shaped gametophytes with a length to width ratio of 2:1.

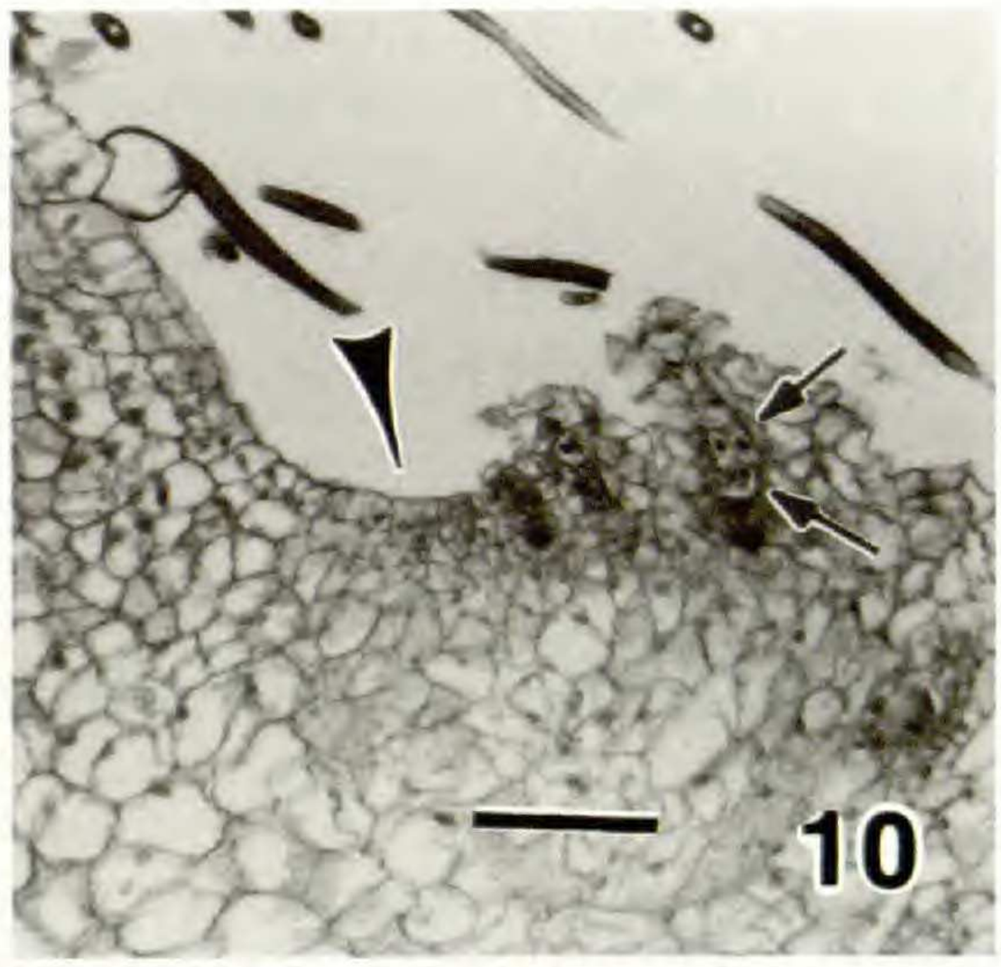
The gametophytes grew in length and width through the activity of the ring meristem (Fig. 1). It formed tissues to the tapering base ventrally and those of the gametangial cap dorsally. Some of the medium-sized gametophytes retained the carrot shape and the 2:1 ratio of length to width (Fig. 1). However, most of the gametophytes of this length and longer were wider (Figs. 2, 3, 4). The 2:1 ratio was lost and it approached a 1:1 ratio in these wider gametophytes.

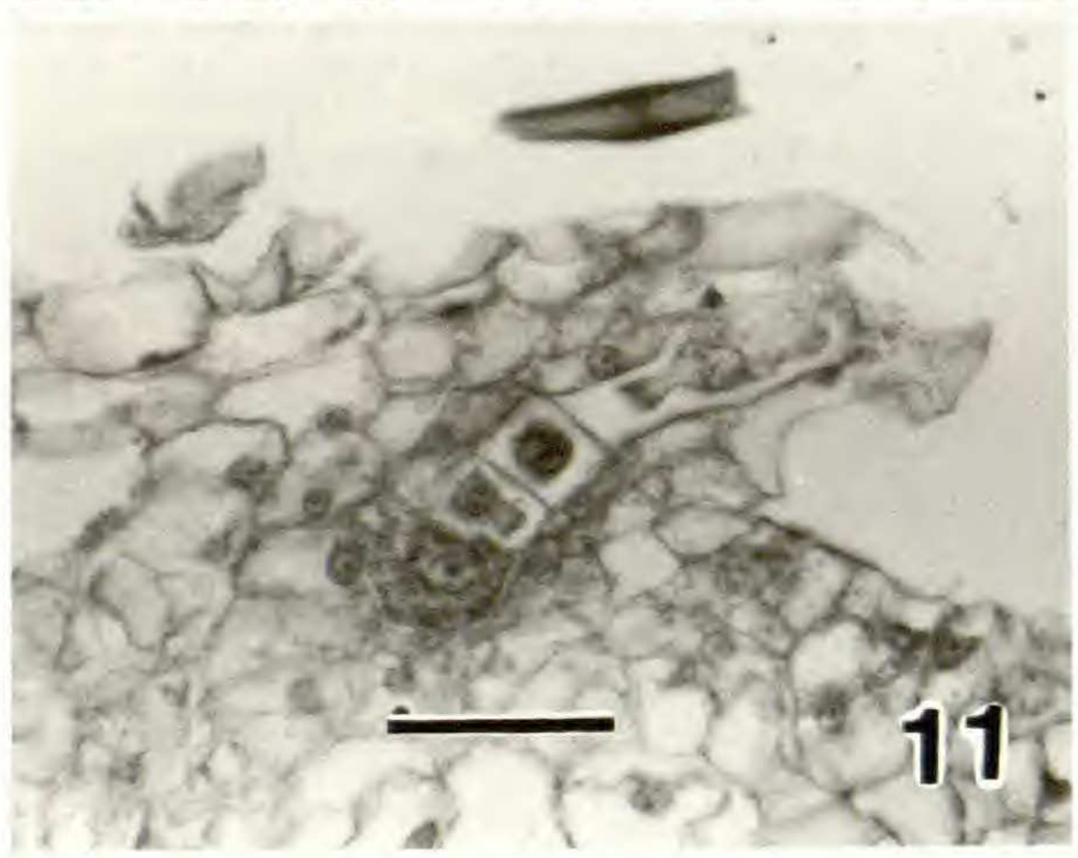
As the ring meristem increased in diameter, the gametangial cap and basal region became larger. The gametophytes increased in width (diameter) with little increase in the length of the basal region. This growth caused the gametophytes to become wider than long (Fig. 5) and the length to width ratio became 1:2. Although the bottom of the gametophyte base remained pointed,

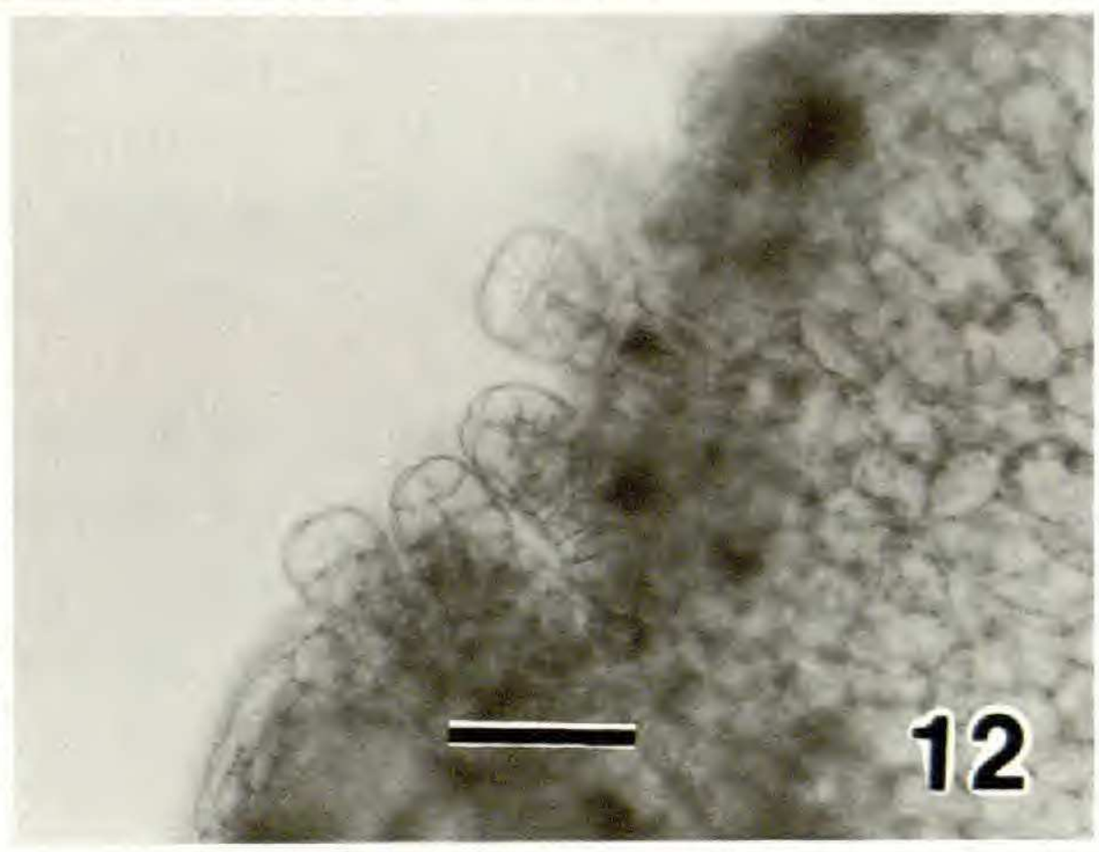


FIGS. 1–8. Gametophytes of *Lycopodium deuterodensum*. 1–3. Lateral views of carrot-shaped gametophytes with gametangial caps, ring meristems (arrows), and tapering bases. 4. Lateral view of wide gametophyte with gametangial cap, ring meristem (arrows), and tapering base. 5. Lateral view of more flattened wider that long gametophyte with ring meristem (arrows). 6. Dorsal view of flattened disk-shaped gametophyte. 7. Ventral view of large irregularly disk-shaped gametophyte. Arrowhead indicates dark tapering base and arrows indicate the position of the ring meristem. 8. Dorsal view of large irregularly disk-shaped gametophyte. Bars = 1 mm for Figs. 1–6 and 5 mm for Figs. 7–8.









FIGS. 9–12. Gametangia of *Lycopodium deuterodensum*. 9. Portion of gametangial cap with developing antheridia. Arrow indicates position of ring meristem. 10. Portion of gametangial cap with archegonia. Arrowhead indicates ring meristem and arrows indicate paired neck canal cells in largest archegonium. 11. Archegonium with egg and 3 neck canal cells. 12. Living archegonia with undisturbed neck cells. Bars = 50 μm for Fig. 11 and 100 μm for Figs. 9, 10 and 12.

the top of the base was larger and flattened. These gametophytes acquired a more flattened condition and were no longer carrot shaped (Figs. 5, 6).

The largest gametophytes formed in culture were thickened with an irregular disk shape (Figs. 7, 8). The ring meristem was located ventrally on the underside of the gametangial cap rather than the lateral position present on smaller gametophytes (Fig. 7). The thickness (height) of the gametangial cap increased and it could overarch the basal region. The original tapering base remained as a small projection on the ventral gametophyte surface (Fig. 7). These thick disk-shaped gametophytes often exceeded 1 cm in diameter.

Antheridia were initiated by the ring meristem and they matured as the new tissues shifted to the edge and top of the gametangial cap (Fig. 9). The mature antheridia were almost completely sunken into the gametangial cap (Fig. 9). At maturity each antheridium contained an ellipsoidal mass of gametes. The average length of the gamete mass was 179 μm and at its widest it had an average

diameter of 89 μm. The antheridia released spermatozoids about 10 minutes after immersing the gametophytes in water. The spermatozoids exuded from the antheridia to a distance of about 80 μm. After a couple of minutes, they swam away. They were solid with a slight twist and were propelled by two flagella.

Rhizoids formed on the gametangial caps in addition to forming on the tapering base. There were short single-celled projections on the surface of the cap. These growths along with the slightly raised areas in association with the antheridia created an irregular surface to the gametangial cap.

Archegonia formed in the same manner as the antheridia through the activity of the ring meristem (Fig. 10). After initiation they moved with the recently formed tissues to the edge and top of the gametangial cap. Mature archegonia (Fig. 11) were found prior to them reaching the edge of the gametangial cap. The use of the paraffin technique to prepare the gametophytes for sectioning caused the terminal neck cells of the archegonia to collapse (Fig. 10, 11). Unopened archegonia with undisturbed neck cells were observed on thick hand sections of living gametophytes (Fig. 12).

The average length of a mature archegonium from base of egg to tip of neck was 119 μm and there were 3–4 cells in the neck canal above the egg. Occasionally, the neck canal cells were binucleate or there were paired neck canal cells (Fig. 10). The neck protruded, on average, 82 μm above the surface of the gametophyte. Some archegonia opened after being immersed in water for several hours. The opening of the archegonia and antheridia suggests that fertilization is a possibility in culture. Unfortunately, flooding gametophytes with water in the culture tubes has not brought about fertilization so far.

Archegonia could not be seen from outside the culture tubes. Examination of gametophytes with some type of microscopy was necessary to observe them. The archegonia were first identified on 18 month old gametophytes. The neck length made the archegonia difficult to recognize on the irregular surface of the gametangial caps. The difficulty in seeing the necks raises the possibility that archegonia were present on gametophytes younger than 18 months.

A collection of fixed gametophytes was used to find the smallest gametophytes with archegonia. Gametophytes about 5 mm in diameter (width) were the smallest found with archegonia. They were slightly smaller than the gametophyte illustrated in Fig. 5. These gametophytes were much wider than long and the archegonia were best observed in the recently formed tissues of the gametangial cap close to the ring meristem.

The ventral portion of the gametophyte, below the ring meristem, was the main area for rhizoid formation. The bulk of this region was composed of the central zone with its large parenchyma cells. At the surface was an epidermal layer that produced the rhizoids. Between the central zone and the epidermal layer were several layers of small, more or less, isodiametric cells. These cells were in the position of the cortex and mycorrhizal zone of Type I and II gametophytes from soil. Absent from these cultured gametophytes were any elongated cells having the same position as the mycorrhizal area of gametophytes from soil.

CONCLUSIONS

The spores of *L. deuterodensum* germinated in dark culture, which is typical for spores from species with mycorrhizal gametophytes. Initial germination some time in the third week is more rapid than has been observed for other spores of the Lycopodiaceae with mycorrhizal gametophytes (Whittier, 1998). The gametophytes of *Lycopodiella* are photosynthetic and spores from some *Lycopodiella* species have the fastest germination for the Lycopodiaceae. The speed of spore germination for *L. deuterodensum* is slightly slower than the fastest germination reported for *Lycopodiella* species, but it is faster than the slower germination of other species of *Lycopodiella* (Whittier, 1998).

These nonphotosynthetic gametophytes grew rapidly and produced antheridia in six months. They had the gametangial cap, ring meristem, and tapering base as described by Bruchmann (1898) for Type II gametophytes of *Lycopodium* (*sensu lato*). The carrot-shaped gametophytes were longer than wide. Their length to width ratio was similar to what has been found in other species with Type II gametophytes from soil (Bruchmann, 1898, 1908; Bruce, 1979) and culture (Whittier, 1981, 2003). At this stage their shape was that of a Type II gametophyte.

As the gametophytes continued to grow, their shape changed. They increased in diameter and became wider so that the length to width ratio shifted from 2:1 to 1:2. These wider gametophytes were the smallest to have both antheridia and archegonia. Thus, the first mature gametophytes in culture were much wider than long and not the typical carrot shape of a Type II gametophyte.

The mature gametophyte of *L. deuterodensum* continued to grow and became flat disk-shaped Type I gametophytes. A shift from a carrot-shaped Type II gametophyte to a disk-shaped Type I gametophyte has not been previously observed. The Type II gametophytes of *L. digitatum* and *L. sitchense* in older cultures continued to grow as carrot-shaped gametophytes (Whittier, 1981, 2003). Whether this late morphological change occurs in nature or is a product of no fertilization in culture is unknown at this time. However, it appears that the Type II gametophyte of *L. deuterodensum* can develop into Type I gametophytes.

The gametangia occur on the gametangial cap as with Type I or II gametophytes. The large sunken antheridia, which produce biflagellate gametes, are essentially the same as those of both Type I and II gametophytes (Bruchmann, 1898; Bruce, 1979; Whittier, 1981, 2003). For the archegonia, both the distance between the base of the egg and tip of the neck and the length of the neck above the gametangial cap are shorter. The number of neck canal cells has been used to distinguish Type I from II gametophytes (Bruchmann, 1898). Bruce (1979) demonstrated that there is much overlap in the numbers of neck canal cells. He found that Type II gametophytes have 9–15 neck canal cells and Type I gametophytes have 3–14. The number of neck canal cells, 3–4, of *L. deuterodensum* better fits the numbers for Type I gametophytes.

Gametophytes grown in culture have a variety of shapes and sizes. All gametophytes old enough to have one or both types of gametangia have a ring meristem, radial symmetry, and lack paraphyses or photosynthetic lobes.

These characteristics, as summarized by Bruce (1979), place the gametophytes of *L. deuterodensum* in the Type I or II grouping.

Archegonia form on gametophytes in culture prior to them becoming flat, disk-shaped gametophytes. However, they are not the typical Type II gametophytes because they are twice as wide as long. These short squat gametophytes are intermediate between Type I and II gametophytes.

Bruce (1979) raised the possibility that gametophytes intermediate between Type I and II may exist in species with unknown gametophytes. After studying many examples, he felt the characteristics that Bruchmann (1898) used to distinguish Type I and II gametophytes from each other were inconsistent. He also noted that the gametophyte of *L. scariosum* has been described as a Type I (Edgerley, 1915; Chamberlain, 1917) and as a Type II (Holloway, 1916). Bruce (1979) suggested that the Type I and II gametophytes be considered a single gametophyte type. The results obtained in this study with *L. deuterodensum* support Bruce's suggestion.

It remains important to collect gametophytes of *L. deuterodensum* from natural areas to conclusively establish the type of gametophyte for this species. Gametophytes from nature with attached sporophytes would indicate the mature gametophyte morphology. If this shape correlates with the size and shape of the first gametophytes in culture with archegonia, it would appear that these gametophytes are intermediate between the typical Type I and II gametophyte. The disk-shaped gametophytes in culture could then be explained by the restriction of fertilization under these conditions. If the gametophytes in nature have sporophytes attached to gametophytes with the size and shape of the last stages found in culture, then the gametophytes of *L. deuterodensum* would have the Type I condition at the time of sexual maturity.

Whatever the gametophyte is like in nature does not obscure the fact that in culture these gametophytes shifted from a young Type II gametophyte to an older Type I gametophyte. Whether this occurs in nature with this species is unknown but the fact that it has happened with *L. deuterodensum* does suggest that gametophytes intermediate between Type I and II probably exist for some *Lycopodium* species. Certainly the results from this study support the suggestion of Bruce (1979) that there may only be one morphologically variable *Lycopodium* gametophyte with a complete ring meristem, radial symmetry, and lacking paraphyses or photosynthetic lobes.

Spore germination and gametophyte development in the dark indicate that the gametophyte of *L. deuterodensum* is subterranean, nonphotosynthetic, and mycorrhizal. The specific type of gametophyte has not been determined by this study. However, there is sufficient evidence to corroborate Holloway's conclusion (1910, 1916) that *L. deuterodensum* has a subterranean gametophyte.

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